A Study of the dosage and duration for levobupivacaine infusion by the caudal-epidural route in infants aged 3-6 months

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Pediatric Anesthesia

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**A Study of the dosage and duration for levobupivacaine infusion by the caudal-epidural route
in infants aged 3-6 months**

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What is already known?

- Levobupivacaine is highly bound to α_1 -acid glycoprotein, an acute phase protein, where unbound levobupivacaine is responsible for toxicity.
- While pharmacokinetics of racemic bupivacaine has been studied for infusions up to 48 hours in neonates and up to 4 hours in infants, pharmacokinetics for levobupivacaine has only been studied after a single shot caudal administration.

What this article adds

- In infants aged 3-6 months, using current recommended dosing schedules of levobupivacaine, total drug concentrations continue to rise during the 48 hour caudal-epidural infusion due to rising α_1 -acid glycoprotein concentrations; however, total drug concentrations remain below suggested toxic concentrations.
- Following major surgery in infants, α_1 -acid glycoprotein levels increase during the first 72 hours.
- Pharmacokinetic modelling shows that unbound levobupivacaine concentrations do not rise beyond the concentrations reached immediately after the loading dose and the unbound drug remains unaffected by α_1 -acid glycoprotein.

Abstract

Aim: To investigate total serum levobupivacaine concentrations after a caudal-epidural loading dose followed by a maintenance infusion in infants aged 3-6 months over 48 hours.

Background: The local anaesthetic, levobupivacaine, is the safer enantiomer of racemic bupivacaine. Present protocols for levobupivacaine are based on studies and pharmacokinetic modelling with racemic bupivacaine. This study will inform clinical practice in this age group and validate the pharmacokinetic model for levobupivacaine infusions in infants, aged 3-6months.

Methods: The clinical trial was conducted in 8 infants aged 3-6 months, undergoing bladder exstrophy repair. Pharmacokinetic modelling allowed optimisation of clinical sampling to measure total levobupivacaine and α_1 -acid glycoprotein and prediction of the effect of α_1 -acid glycoprotein on levobupivacaine plasma protein binding.

Results: The observed median total levobupivacaine serum concentration was 0.30 mg.L⁻¹ (range: 0.20-0.70 mg.L⁻¹) at 1 hour after the loading dose of 2 mg.kg⁻¹. The median total levobupivacaine concentration after 47 hours of infusion, at 0.2 mg.h⁻¹.kg⁻¹, was 1.21 mg.L⁻¹ (0.07-1.85 mg.L⁻¹). Concentrations of α_1 -acid glycoprotein were found to rise throughout the study period. Pharmacokinetic modelling suggested that unbound levobupivacaine quickly reached steady state at a concentration of approximately 0.03 mg.L⁻¹.

Conclusion: This study examines the pharmacokinetics of levobupivacaine after a loading dose (given over 5 minutes) followed by a maintenance infusion in infants 3-6 months. The study allows the development of a pharmacokinetic model, combining levobupivacaine and α_1 -acid glycoprotein data. Modelling indicates that unbound levobupivacaine quickly reaches steady state once the infusion is started. Simulations suggest that it may be possible to continue the infusion beyond 48 hours.

Keywords:
levobupivacaine; α_1 -acid glycoprotein; infants; caudal-epidural infusion; bladder exstrophy; pharmacokinetics

List of abbreviations:
AAG: α_1 -acid glycoprotein
LBU: levobupivacaine
CYP: cytochrome P450
 CL_u : unbound clearance
F: bioavailability
 k_a : rate of absorption
 R_0 : rate of infusion
 V_u : unbound volume of distribution
 K_D : dissociation constant
 $C_{tot, LBU}$: Concentration of total levobupivacaine
 $C_{u, LBU}$: Concentration of unbound levobupivacaine

Background
Levobupivacaine is the S (-) enantiomer of racemic bupivacaine, displaying a lower risk of cardiovascular and central nervous system toxicity while still retaining local anaesthetic potency.¹ In paediatric patients, the elimination increases with age and reaches 80% of the adult capacity by the age of 6 months.² Present protocols of caudal-epidural infusions of levobupivacaine are based upon studies of bupivacaine and pharmacokinetic modelling.^{3,4,5} Previous studies of levobupivacaine are limited to examining serum concentrations of levobupivacaine in infants following a single caudal-epidural bolus dose.^{6,7,8} A bolus dose followed by an infusion for 24 hours has been studied in infants over 6 months.⁹ At

the same time, studies of racemic bupivacaine in infants¹⁰ and neonates¹¹, with infusion periods of 12 and 48 h, respectively, have raised concerns over its safety in this age group.⁴

A study of serum concentrations of levobupivacaine after a loading dose followed by an infusion for 48 hours, in infants aged 3-6 months, would help inform clinical practice. By restricting the age group we focus on the maturation of the liver and clearance with age in these infants. Since the unbound local anaesthetic is responsible for toxicity, the degree of binding to the acute phase protein, α_1 -acid glycoprotein (AAG), should be considered.

Bladder Exstrophy is a rare congenital abnormality, with an incidence of 1:45,500¹², requiring major corrective surgery in early infancy. This involves closure of the bladder and abdominal wall. The splayed pubic bones are brought together by an anterior osteotomy and an external pelvic fixator remains *in situ* for 4-6 weeks. Surgery takes 6-8 h and infants can be extubated at the end of the procedure if a caudal-epidural catheter has been sited and an infusion of levobupivacaine provides postoperative pain. At present clinicians are limited by the volume, concentration and duration of the infusions due to concerns regarding cardiovascular and central nervous system toxicity.

Here we present a clinical trial examining serum concentrations of total levobupivacaine and AAG in this group of patients. Aims:

- To use pharmacokinetic analysis to predict the unbound levobupivacaine concentrations for the study protocol
- To use pharmacokinetic modelling and consider the safety of alternative dosing schedules e.g. increasing the loading dose and an earlier onset of the maintenance infusion.

Methods

This study was conducted as a Phase II/III clinical trial (EudraCT no: 2015-000393-34) with the MHRA (21387/0235/001-0001) and Northwest Ethics Committee approval (15/NW/0240). The trial was conducted in compliance with the protocol, following GCP guidelines. Written informed consent was obtained from parents.

The study population consisted of infants aged three to six months undergoing repair of Bladder Exstrophy.

The exclusion criteria were lack of consent, hypersensitivity to local anaesthetics, history of seizures or neurological disorders, bradyarrhythmias, blood clotting disorders, congenital abnormalities precluding regional anaesthesia.

The trial was designed in several stages. A literature-based joint LBU and AAG pharmacokinetic model was developed to predict the impact of changes of AAG concentrations, associated with surgery, on total levobupivacaine

concentrations. Prospective pharmacokinetic simulations of levobupivacaine and AAG were carried out in Matlab R2012a (Mathworks, Natick, MA) to inform the blood sampling strategy. This was based on a population pharmacokinetic model of unbound levobupivacaine following a caudal injection of levobupivacaine 2 mg.kg^{-1} given over 30 seconds, in infants undergoing sub-umbilical surgery.⁸ The model was modified to account for time-dependent changes in levels of AAG following surgery.¹⁴ It allowed the estimation of optimal sampling time points, limiting blood sample volumes in accordance with the WHO guidance.¹⁵ Optimal design evaluations were performed using PopDes(The University of Manchester; Matlab: <http://research.bmh.manchester.ac.uk/capkr/softwaretools/>) based on the preliminary model.¹⁶

Infants were recruited over a period of 24 months (June 2015 - June 2017). The clinical data collected were then used to develop a novel population pharmacokinetic model of levobupivacaine and AAG to simulate alternative dosing protocols.

Conduct of the study

At preoperative assessment a week prior to surgery, details of the planned anaesthetic and clinical trial were discussed and a written information sheet provided. Consent was obtained on the morning of surgery. Anaesthesia was induced with sevoflurane in oxygen and atracurium 0.5 mg.kg^{-1} was given to facilitate orotracheal intubation. Anaesthesia was maintained using sevoflurane in oxygen and air through a circle system. Invasive arterial monitoring and a central venous line (4 Fr, 5 cm double lumen) were established. An Oesophageal Doppler cardiac output monitor (Deltex Medical) was also used to assist fluid management. The caudal space was imaged using ultrasound and an Arrow, flexitip® reinforced catheter was inserted. This was tunnelled to the right flank and the catheter tip position was confirmed using fluoroscopy.

Caudal Epidural Dosing Regime

A loading dose of levobupivacaine $0.25\% 2 \text{ mg.kg}^{-1}$ (0.8 mL.kg^{-1}) was injected over 5 minutes (start of loading dose was time zero). A maintenance infusion of levobupivacaine $0.125\% 0.2 \text{ mg.kg}^{-1}.\text{h}^{-1}$ with clonidine (1.5 mcg.mL^{-1})¹⁷ was started one hour after time zero and run for 47 h.¹¹ All infants also received a remifentanyl infusion (25 mcg.mL^{-1}) intraoperatively which was titrated as required. Additional intraoperative medications included paracetamol (10 mg.kg^{-1})

¹), ondansetron (0.1 mg.kg⁻¹) and dexamethasone (0.1 mg.kg⁻¹). Plasmalyte 148 was used as the intraoperative fluid and arterial blood gas results were used to monitor blood sugar levels. Infants were extubated at the end of surgery and managed in the paediatric critical care unit (PCCU) while the caudal-epidural catheter was still *in situ*. Regular postoperative analgesia was prescribed: intravenous paracetamol 10 mg.kg⁻¹ every 6 h and per oral ibuprofen 5mg.kg⁻¹ every 8 h. Oral morphine 50 mcg.kg⁻¹, 4 hourly was prescribed for FLACC > 4. Post-operative pain scores were noted hourly (FLACC scale: face, legs, activity, cry, consolability). The caudal-epidural infusion was stopped after 47 hours and blood samples were collected for analysis up to 72 hours after the loading dose.

Blood sampling

Blood samples were taken from the indwelling central venous catheter. Dead space sampling was performed and the volume from the dead space was injected back using antiseptic non-touch technique. No intravenous fluids were administered through the central line. The samples were collected in plain glass pyrex tubes for levobupivacaine (2mL/sample) and lithium heparin for AAG (0.8mL/sample). The samples were labelled 1 to 9 with sampling time points at 0.25, 1, 2, 4, 12, 36, 48, 60 and 72 hours. (Table S1).

Blood Sample Management

All samples were anonymised for analysis, identifiable only by a unique number to allow data linkage. Blood samples were stored at 4°C then centrifuged in the laboratory. The serum was then stored at -80°C.

Analysis of levobupivacaine was undertaken in a CPA accredited laboratory (Department of Clinical Pathology, Queens Medical Centre, Nottingham) where they were centrifuged at 2,500 rpm for 10 minutes. Levobupivacaine was analysed in a matrix of human plasma using a developed and validated gas chromatography-mass spectrometry (GC-MS) approach. Levobupivacaine measurements were made according to published methods.^{18,19} 0.5 mL of serum was alkalinised with sodium hydroxide with extraction of levobupivacaine into diethyl ether. The extract was then evaporated to dryness under nitrogen prior to reconstitution in methanol and analysis by GC-MS. The lower limit of quantification (LLOQ) of the assay was 0.1 mg.L⁻¹ and the linear range of the assay without prior dilution was 10 mg.L⁻¹.

Ropivacaine was used as the internal standard. The AAG samples were analysed in the biochemistry laboratory at Royal Manchester Children's Hospital.

Statistical analysis

This study was conducted as a Phase II/III clinical trial. It was designed as an exploratory observational study to develop the pharmacokinetic model in this age group with an aim to be used to inform further studies. It aimed to recruit 10 infants over a 2 year period, with a final sample of 8 infants. All parents approached, consented to participate in the trial. Descriptive statistics and graphs were used to explore the serum concentration-time profiles and the between patient variability. Rv3.0.2 software (<https://www.r-project.org/>) was used for this descriptive and graphical analysis.²⁰

Data management

All data were anonymised and only identifiable by a unique study number allocated at recruitment. Data collection was performed by one of the investigators and stored in both paper and electronic form.

Pharmacokinetic Analysis

Population pharmacokinetic modelling was carried out to analyse the generated clinical data (total levobupivacaine and AAG) from 8 infants. This was performed using NONMEM 7.3 (ICON plc, Dublin, Ireland). Identifiability analysis of the proposed AAG and levobupivacaine models was carried out using the software DAISY (Differential Algebra for Identifiability of Systems; University of Cagliari, Cagliari, Italy)²¹ and the models were found to be globally identifiable. Due to the limited dataset (n patients=8), covariate analysis was not considered. Data below the LLOQ were included in the analysis (7 out of a total 66 data points were used in analysis, or 10.6%) as per the all data approach recommended by Keizer *et al.*^{22,23}

In one of the infants (infant 3), the levobupivacaine infusion was stopped after approximately 12 h. Sampling points beyond 12 h were excluded.

The AAG model was developed to describe the time-dependent change in AAG levels following surgery. This was followed by the development of the population pharmacokinetic model to describe total levobupivacaine concentrations following continuous caudal-epidural infusion. Diagrams of the pharmacokinetic models are displayed in Figure 1.

The levobupivacaine pharmacokinetic model was parameterised to describe the unbound parameters (k_a , CL_u/F and V_u/F), as the observed data indicated time-dependent variations in pharmacokinetic parameters based on total concentrations. Due to the inability to measure unbound levobupivacaine in this study, total concentrations were derived using the post-hoc individual parameter estimates for the AAG model together with a calculated K_D (plasma protein binding dissociation constant) with a geometric mean of 32.6 mg.L⁻¹ (geometric standard deviation: 1.1 mg.L⁻¹) derived

from adult data digitised from Veering *et al.*²⁴ Digitisation was carried out using GetData Graph Digitizer (S. Federov; see Supplementary Material for further information).

Model performance was evaluated by examining visual predictive checks (VPCs, number of simulations=10,000) and goodness-of-fit (GOF) plots (Figure S2, Supplementary Material). Alternative dosing strategies were simulated in Matlab using the final model.

Results

Eight infants aged 3-6 months (mean age: 5 months, standard deviation (SD): 0.5 months, range: 4 – 5.9 months at the time of surgery) were recruited over a period of 24 months. There were five boys and three girls. Mean bodyweight was 6.90 kg (SD: 0.96, range: 5.97-8.67 kg). There were no associated congenital abnormalities other than bladder exstrophy and biochemistry was normal. A caudal-epidural loading dose (short infusion over 5 minutes) was given followed 60 minutes later by a 47 h maintenance infusion. The effect of the acute phase protein, AAG, on the serum concentrations of total levobupivacaine was investigated. Complete data were available for 6 out of 8 (75%) infants. For one of the infants (infant 5), samples at 60h and 72h could not be obtained due to a blocked central line. Another infant (infant 3) had their caudal catheter removed at 12h as they developed respiratory distress and high temperature and needed to be re-intubated. The FLACC pain scores for all the infants remained below 4 during the trial period. All infants received a blood transfusion, mean volume of 13.4 mL.kg⁻¹ (range of 60 – 130 mL) approximately between 2 h and 4 h. Intraoperative fluids used were Plasmalyte 148, Plasmalyte with Dextrose 5% if required and Gelofusine. No Albumin was used. Gelofusine: mean of 12.4 mL.kg⁻¹ (range 60-130 mL), crystalloid mean of 64.2 mL.kg⁻¹ (range 395 -504 mL).

The results from the analysis of total levobupivacaine concentrations at 1 h showed a median total serum concentration of 0.3 mg.L⁻¹ (range: 0.20-0.70 mg.L⁻¹) after the 5 minute short infusion loading dose of 2mg.kg⁻¹. The median total levobupivacaine concentration after 47 h of the maintenance infusion (0.2 mg.kg⁻¹.h⁻¹) was 1.21 mg.L⁻¹ (range: 0.07-1.85 mg.L⁻¹) (Figure 2). Total levobupivacaine concentrations increased throughout the infusion period and serum AAG concentrations continued to rise up to 72 h (Figure 3). This showed consistency with the predicted plasma concentration-time profiles that were simulated prospectively to inform the study design (Figure S2, Supplementary Material). The final AAG model was modified from a Hill function to an empirical precursor model with an interaction term to best describe the observed data in this study (Figure 1; Equations S2 and S3 Supplementary Material, and Table1). The population

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3 pharmacokinetic analysis based on unbound parameterisation showed levobupivacaine to display one compartmental
4 disposition (Figure 1; Equations S4-S6, Supplementary Material) with slow release from the site of administration to the
5 systemic circulation with an estimated absorption rate constant (k_a) of 0.15 h^{-1} . The average apparent unbound clearance
6 (CL_u/F) and apparent unbound volume of distribution (V_u/F) were $61.30\text{ L}\cdot\text{h}^{-1}$ and 1.05 L , respectively (Table 1). Figures
7 2 and 3 show the visual predictive checks (number of simulations=10,000) for total levobupivacaine and AAG over time,
8 respectively.

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16 Pharmacokinetic modelling suggested that unbound levobupivacaine levels quickly reached steady state concentrations
17 after the short infusion, loading dose of levobupivacaine 0.25% ($2\text{ mg}\cdot\text{kg}^{-1}$). The simulated unbound levels were then
18 maintained throughout the infusion period at around $0.03\text{ mg}\cdot\text{L}^{-1}$ (Figure 4).

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21 We also simulated an alternative dosing protocol which is the same as that used in infants over 6 months ($2.5\text{ mg}\cdot\text{kg}^{-1}$
22 loading dose) followed immediately by a maintenance infusion of $0.2\text{ mg}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$). Simulated concentration-time profiles
23 (Figure 5) using this alternative dosing were very similar to the standard protocol and showed only a slight increase in
24 unbound concentrations (remaining below $0.08\text{ mg}\cdot\text{L}^{-1}$).

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32 **Discussion**

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34 This clinical trial investigates serum concentrations of the local anaesthetic levobupivacaine in infants aged 3-6 months.
35 Other studies^{5,7,8,10,11} have been limited due to the difficulty in obtaining serial samples from infants over a prolonged
36 time period. Due to these limitations clinical questions regarding the concentration of the loading dose and timing of the
37 maintenance infusion have so far remained unanswered and our study attempts to answer these questions.

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43 We chose to conduct this study in infants with bladder exstrophy who require major corrective surgery for the
44 abnormality at an early age. Central venous access is routinely used for monitoring and was used to obtain blood
45 samples. A caudal-epidural catheter is also recommended for pain relief.²⁵ These patients may potentially benefit from
46 higher doses of local anaesthetic, particularly at the time of the osteotomy. However, clinicians are limited by existing
47 dosing protocols due to the possibility of central nervous system and cardiovascular side effects, with higher doses and
48 longer duration of the infusion in infants. Choosing our particular group of infants with a rare congenital abnormality (5-
49 7 infants at our centre per year) limited recruitment to only 8 infants.

The small number of study participants prohibited any investigation of covariate associations with pharmacokinetic parameters and resulted in a potential exaggeration of variability in the simulated AAG and levobupivacaine concentration levels. This is not uncommon when analysing datasets consisting of a small number of study participants.

Levobupivacaine is extensively metabolised by cytochrome P450 (CYP) 3A4 and 1A2.^{1,2} Chalkiadis *et al.* found bodyweight, normalised clearance of levobupivacaine to increase following birth and approach 80% of adult values at six months of age.²

Our results showed that the serum concentration of total levobupivacaine increased after the initial loading dose and continued to rise throughout the infusion period. When the infusion was stopped the levels fell rapidly (Figure 2). This confirms findings by Larsson *et al.* with bupivacaine in neonates receiving epidural infusions.¹¹

Concentrations of AAG were shown to increase during the course of the surgery and appeared to plateau towards the end of the study period, which is consistent with previous studies.²⁴ The magnitude of increase in AAG observed in this study is larger (approximately a 4-fold median increase) compared to that observed by Booker *et al.* (approximate 2-fold increase)²⁶ and in the model developed by Aarons *et al.*¹⁴ As a result of rich sampling at early time points we observed a minor but consistent reduction in AAG concentrations in the immediate hours after the levobupivacaine, loading dose. This can be best described using an empirical precursor model, inhibiting AAG formation and simultaneously inducing precursor formation. This provides a reasonable description of the observed data. Care should be taken not to make inferences about the underlying mechanism based on this model.

Levobupivacaine displays linear binding to AAG, binding around 97%.¹ Concentration-dependent plasma protein binding with low to moderate hepatic extraction drugs such as levobupivacaine, will cause variation in total drug concentrations and associated pharmacokinetic parameters, whereas unbound concentrations and parameters will remain unaffected.⁸

Ideally, the unbound drug concentration should be measured as it drives pharmacological and toxicological effect. The inability to measure these data is an apparent limitation of the current study. However, a study protocol was devised to allow indirect inference of unbound levobupivacaine concentrations and unbound pharmacokinetic parameters by incorporating information on total levobupivacaine and AAG concentrations, and K_D , based on published adult data.²⁴

The developed model was successful in describing the time-dependent change in total levobupivacaine concentrations due to changes in AAG and gave an indication of the unbound levobupivacaine concentration-time profile. Estimated CL_u/F was higher and V_u/F was lower compared to that reported by Chalkiadis *et al.*⁸ This may be due several factors, including age differences of study participants in the two clinical trials (ontogeny of clearance)² and differences in study duration (4 h versus 72 h here). Further, the approach detailed here is susceptible to uncertainty in the determination of K_D and its ontogeny.²⁴ Aarons *et al.* found K_D of ropivacaine plasma protein binding to vary with age based on population pharmacokinetic analysis of unbound and total drug concentration-time profiles.¹⁴ It is therefore plausible that the AAG K_D of levobupivacaine also may vary with age.

The pharmacokinetic model suggests that the unbound concentration of levobupivacaine quickly reaches steady state concentrations following a loading dose and is maintained at the same level for the duration of the infusion at the current rate. This is an average concentration of 0.03 mg·L⁻¹ of unbound levobupivacaine throughout the infusion period. We also simulated an alternative dosing protocol (2.5 mg·kg⁻¹ loading dose followed immediately by a maintenance infusion of 0.2 mg·kg⁻¹·h⁻¹). Simulated concentration-time profiles (Figure 5) using this alternative dosing were very similar to the standard protocol and showed only a slight increase in unbound concentrations (remaining below 0.08 mg·L⁻¹). This protocol could reduce the need for opioid rescue analgesia in this age group when receiving a caudal-epidural, which is sometimes, required with the present local anaesthetic dosage limitations. However, further studies are needed to confirm this. Evidence suggests keeping serum total bupivacaine levels below 2-2.5 mg·L⁻¹ to avoid toxicity.⁴ Levobupivacaine has an improved safety profile^{1,6,7,8} but there are no studies indicating unbound serum levobupivacaine toxic levels.

Some of the individual profiles indicated complex absorption kinetics from the epidural space. This would perhaps be best described using a combined slow and fast absorption rate as previously shown through modelling of epidural levobupivacaine in adults.²⁷ The complex absorption was however only apparent in a small proportion of study participants and not significant enough to allow inclusion in the current model.

All infants required blood transfusion. The effect of blood transfusion is rarely considered in pharmacokinetic analysis and little is known about its impact on drug disposition. Although theoretically possible, no apparent trends were seen when comparing blood transfusion volumes to estimated individual pharmacokinetic parameters.

Clonidine is a common adjuvant to levobupivacaine in a single dose or caudal infusions. Clonidine was a non-investigational medicinal product (NIMP) in this clinical trial and was used in a concentration of 1.5 mcg·mL⁻¹ based on

previous studies.^{13,17} Chalkiadis *et al.*¹⁷ indicated that levobupivacaine is more rapidly absorbed from the caudal-epidural space when clonidine is added. While we were not able to compare levobupivacaine absorption pharmacokinetics with and without clonidine, total serum concentrations of levobupivacaine were well below suggested toxic levels throughout the study.

One of the infants (infant 3) had to be excluded from data analysis as this infant was reintubated and caudal catheter removed at 12 h due to high grade pyrexia and respiratory distress. This was recorded as a serious adverse event (SAE). Viral and bacterial titres were negative and the infant was extubated after 48 hours. Pain scores, remained below 4 (FLACC) in all infants using prescribed rescue analgesia.

Conclusions

This trial has allowed the development of a pharmacokinetic model for the studied age group combining levobupivacaine and AAG data. The simulated concentration-time profiles show that unbound levobupivacaine concentrations reach an early steady state level following the loading dose and the simulated steady state concentrations are below suggested toxic concentrations. Hence it is possible to increase the duration of the infusion to more than 48 hours.

The current dosing recommendations may be too conservative when using levobupivacaine in the studied age group. However, over a broader age range, variation in elimination or a compromised liver function must be taken into consideration.

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Declaration of Interests

None to declare.

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Table:

Parameter	Estimate	%BSV	95%CI
AAG parameters			
CL ₀ (L.h ⁻¹)	0.136	-	0.125, 0.141
C(0) (mg.L ⁻¹)	413	31.2	292, 475
INT	4.58	54.6	3.50, 5.13
Err prop (%)	7.81	-	4.25, 14.34
Levobupivacaine parameters			
k _a (h ⁻¹)	0.15	-	0.138, 0.156
V _u /F (L)	1.05	-	0.62, 1.27
CL _u /F (L.h ⁻¹)	61.3	28.1	48.1, 68.0
K _D (mg.L ⁻¹)	32.6	-	fixed
Err prop (%)	33.0	-	12.15, 89.72

CL₀ = **conversion clearance**; C(0) = concentration of AAG and pre-AAG at time zero; INT = interaction term; k_a = first-order absorption rate; V_u/F = apparent unbound volume of distribution; CL_u/F = apparent unbound clearance; BSV = interindividual variability presented as %CV (coefficient of variation); Err prop = proportional residual variability; 95% CI are the 95% confidence intervals.

Table 1: α₁-acid glycoprotein (AAG) dynamic model and levobupivacaine pharmacokinetic model, final parameter estimates.

LEGENDS FOR FIGURES

Figure 1. Schematic of final model. The model describes time-dependent changes in total levobupivacaine ($C_{\text{tot,LBU}}$) as dependent on unbound levobupivacaine concentrations ($C_{\text{u,LBU}}$), α_1 -acid glycoprotein (C_{AAG}) and the binding isotherm (k_D). R_0 =rate of infusion, k_a =absorption rate, CL_u =unbound apparent clearance, CL_0 =formation clearance of pre-AAG, conversion clearance from pre-AAG, elimination clearance of AAG. $C_{\text{Pre-AAG}}$ =AAG precursor.

Figure 2. Measured levels of total levobupivacaine concentration ($C_{\text{tot,LBU}}$) over time. Visual predictive check of total levobupivacaine concentration over time. Observed data indicated by (o), observed median data indicated by (--), median, 95th and 5th percentiles of model simulations indicated by (-) and grey area.

Figure 3. α_1 -acid glycoprotein (AAG) concentration (C_{AAG}) over time. Visual predictive check of AAG concentration over time. Observed data indicated by (o), observed median data indicated by (--), median 95th and 5th percentiles of model simulations indicated by (-) and grey area

Figure 4. Simulated total (A) and unbound (B) concentration-time profiles of levobupivacaine ($C_{\text{tot,LBU}}$ and $C_{\text{u,LBU}}$, respectively) at the current dosing schedule. C: simulated α_1 -acid glycoprotein concentration (C_{AAG}) over 72 hours. Median, 95th and 5th percentiles of model simulations indicated by (-) grey area.

Figure 5. Simulated total (A) and unbound (B) concentration-time profiles of levobupivacaine ($C_{\text{tot,LBU}}$ and $C_{\text{u,LBU}}$, respectively) at an alternative dosing protocol. C: α_1 -acid glycoprotein concentration over 72 hours. Median, 95th and 5th percentiles of model simulations indicated by (-) and grey area.

Figure 1. Schematic of the final model. The model describes time-dependent changes in total levobupivacaine ($C_{tot,LBU}$) as dependent on unbound levobupivacaine concentrations (C_u,LBU), α 1-acid glycoprotein ($CAAG$) and the binding isotherm (k_D). R_0 =rate of infusion, k_a =absorption rate, CL_u =unbound apparent clearance, CL_0 =formation clearance of pre-AAG, conversion clearance from pre-AAG, elimination clearance of AAG. $C_{Pre-AAG}$ =AAG precursor.

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Figure 2. Measured levels of total levobupivacaine concentration ($C_{\text{tot,LBU}}$) over time. Visual predictive check of total levobupivacaine concentration over time. Observed data indicated by (o), observed median data indicated by (---), median, 95th and 5th percentiles of model simulations indicated by (-) and grey area.

96x63mm (300 x 300 DPI)

Figure 3. α 1-acid glycoprotein (AAG) concentration (C_{AAG}) over time. Visual predictive check of AAG concentration over time. Observed data indicated by (o), observed median data indicated by (---), median, 95th and 5th percentiles of model simulations indicated by (-) and grey area

111x83mm (300 x 300 DPI)

Figure 4. Simulated total (A) and unbound (B) concentration-time profiles of levobupivacaine ($C_{tot,LBU}$ and $C_{u,LBU}$, respectively) at the current dosing schedule. C: simulated α 1-acid glycoprotein concentration (C_{AAG}) over 72 hours. Median, 95th and 5th percentiles of model simulations indicated by (-) grey area.

176x185mm (300 x 300 DPI)

Figure 5. Simulated total (A) and unbound (B) concentration-time profiles of levobupivacaine ($C_{tot,LBU}$ and $C_{u,LBU}$, respectively) at an alternative dosing protocol. C: α 1-acid glycoprotein concentration over 72 hours. Median, 95th and 5th percentiles of model simulations indicated by (-) and grey area.

176x185mm (300 x 300 DPI)

Supplementary material

A Study Of The Dosage And Duration For Levobupivacaine Infusion By The Caudal-Epidural Route In Infants Aged 3-6 Months

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Determining the dissociation constant for levobupivacaine binding to α_1 -acid glycoprotein

The plasma protein binding dissociation constant (K_D) between levobupivacaine (LBU) and α_1 -acid glycoprotein (AAG) was determined based on the measured fraction unbound in plasma (f_{u_p}) and AAG concentrations (C_{AAG}) reported in eight adult patients as per Veering *et al.*, 2002 (Figure 2 in Veering *et al.*, 2002)¹. The data was digitised using GetData Digitizer and analysed in Microsoft Excel. K_D was calculated for each individual data point based on Equation S1, where C_u is the measured unbound concentration and C the total concentration of LBU, and C_{AAG} is the concentration of AAG. The geometric mean K_D was determined to be 32.6 mg.L⁻¹ with a geometric standard deviation of mg.L⁻¹. Using the determined K_D , a mean absolute error (MAE) of 0.7 was given (Figure S1).

$$C_u = C \cdot \frac{1}{1 + \frac{C_{AAG}}{K_D}}, \text{ where } f_{u_p} = \frac{C_u}{C}, K_D = -\frac{f_{u_p} \cdot C_{AAG}}{f_{u_p} - 1}$$

Equation S1

Prospective prediction of levobupivacaine

Figure S2 shows bodyweight, age and dose-matched simulations of average AAG, total and unbound levobupivacaine concentrations ($C_{tot,LBU}$ and $C_{u,LBU}$, respectively) using the prospectively developed model based on Chalkiadis *et al.*, 2005² and Aarons *et al.*, 2000³ to inform optimal design of the clinical study sampling time points.

Model equations for α_1 -acid glycoprotein and levobupivacaine

The final AAG-LBU model is presented in Equations S2-S6. The AAG model that produced the best fit to the observed AAG data, meaning it was able to describe the initial reduction and subsequent increase in AAG concentration, was a precursor model with positive feedback. The model described time dependent concentrations of a theoretical precursor of AAG ($C(t)_{pre-AAG}$; Equation S2) and AAG ($C(t)_{AAG}$; Equation S3). The production and elimination clearances of AAG and its precursor were equal to CL_0 , meaning these clearances rates were assumed to be equal across the two states and at time zero the initial concentrations of $C(0)_{pre-AAG}$ and $C(0)_{AAG}$ were set to equal C_0 . Further, a static interaction constant (INT) was estimated, meaning it did not vary over time. INT acted in the model by inhibiting pre-AAG conversion to AAG and simultaneously inducing pre-AAG production at the same magnitude throughout the study time period. Prior to parameter estimation of CL_0 , C_0 and INT , the model was evaluated in the software DAISY to test global identifiability. **The model developed for the AAG data should be viewed as an empirical description of the AAG levels and no mechanistic interpretation is implied.**

$$\frac{dC_{pre-AAG}}{dt} = C_0 \cdot CL_0 \cdot INT - C(t)_{pre-AAG} \cdot CL_0 / INT \quad C(t=0)_{pre-AAG} = C_0$$

Equation S2

$$\frac{dC_{AAG}}{dt} = C(t)_{pre-AAG} \cdot CL_0 / INT - C(t)_{AAG} \cdot CL_0 \quad C(t=0)_{AAG} = C_0$$

Equation S3

Unbound amount of LBU ($A(t)_{u,LBU}$) was modelled using a one-compartment model with first-order absorption rate (k_a) from the dosing compartment ($A(t)_{LBU-depot}$) with linear elimination (CL_u/V_u). Unbound levobupivacaine concentrations ($C(t)_{u,LBU}$) were then converted to total concentrations ($C(t)_{tot,LBU}$) using an assumed linear relationship (K_D) based on adult data as per Veering *et al.* 2000¹ (Equations S4-S6). In Equation 6, is shown the simplification of the conversion of $C(t)_{u,LBU}$ to $C(t)_{tot,LBU}$ when assuming $C_{u,LBU}$ to be significantly smaller as compared to K_D .

$$\frac{dA_{LBU,depot}}{dt} = -k_a \cdot A(t)_{LBU,depot}$$

Equation S4

$$\frac{dA_{u,LBU}}{dt} = k_a \cdot A(t)_{LBU,depot} - A(t)_{u,LBU} \cdot \frac{CL_u}{V_u} \quad C(t)_{u,LBU} = \frac{C(t)_{u,LBU}}{V_u}$$

Equation S5

$$C(t)_{tot,LBU} = C(t)_{u,LBU} \cdot \left(1 + \frac{C(t)_{AAG}}{C(t)_{u,LBU} + K_D}\right), C_{u,LBU} \ll K_D, C(t)_{tot,LBU} = C(t)_{u,LBU} \cdot \left(1 + \frac{C(t)_{AAG}}{K_D}\right)$$

Equation S6

Final model parameter estimates

Final model parameter estimates are given in for the AAG model, in Table S1, and for the levobupivacaine model in Table S2.

Goodness-of-fit plots

Goodness-of-fit plots for the AAG and levobupivacaine models are shown in Figure S3.

References

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Tables:

Time (hours)	0.25	1	2	4	12	36	48	60	72
AAG: 0.8 mL per sample	1	1	1	1	No sample	1	No sample	1	1
LBU: 2mL per sample	1	1	1	1	1	1	1	1	1

Table S1: Blood sampling schedule for levels of α_1 -acid glycoprotein (AAG) and levobupivacaine (LBU).

Figure Legends

Figure S1. Fraction unbound levobupivacaine in plasma (f_{up}) against concentration of α_1 -acid glycoprotein (C_{AAG}). Observed data (o) digitised from Veering *et al.*, 2002 (1), dashed line (--) indicate model prediction at $K_D=32.6 \text{ mg.L}^{-1}$. K_D : geometric mean plasma protein binding dissociation constant, GSD: geometric standard deviation, MAE: mean absolute error

Figure S2. Simulated and observed levobupivacaine (LBU) and α_1 -acid glycoprotein (AAG) concentration-time profiles based on prospective predictions using the model intended for optimal design. **A:** Simulated unbound plasma concentration of levobupivacaine ($C_{u,LBU}$), **B:** Simulated (-) and observed (o) total plasma concentration of levobupivacaine ($C_{tot,LBU}$), **C:** simulated (-) and observed (o) AAG levels (C_{AAG}), **D:** Simulated fraction unbound (f_{up}) of levobupivacaine over time.

Figure S3. Goodness-of-fit plots for α_1 -acid glycoprotein (A, C, E) and total levobupivacaine (B, D, F), respectively.

Figure S1. Fraction unbound levobupivacaine in plasma (f_{u_p}) against concentration of α_1 -acid glycoprotein (C_{AAG}). Observed data (o) digitised from Veering *et al.*, 2002 (1), dashed line (--) indicate model prediction at $K_D=32.6 \text{ mg} \cdot L^{-1}$. K_D : geometric mean plasma protein binding dissociation constant, GSD : geometric standard deviation, MAE : mean absolute error

111x83mm (300 x 300 DPI)

Figure S2. Simulated and observed levobupivacaine (LBU) and α_1 -acid glycoprotein (AAG) concentration-time profiles based on prospective predictions using the model intended for optimal design. A: Simulated unbound plasma concentration of levobupivacaine ($C_{u,LBU}$), B: Simulated (-) and observed (o) total plasma concentration of levobupivacaine ($C_{tot,LBU}$), C: simulated (-) and observed (o) AAG levels (C_{AAG}), D: Simulated fraction unbound (f_{up}) of levobupivacaine over time

199x162mm (300 x 300 DPI)

Figure S3. Goodness-of-fit plots for α_1 -acid glycoprotein (A, C, E) and total levobupivacaine (B, D, F), respectively.

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